

CLAIMS

1. A genetically modified lymphoid cell having gene conversion fully or partially replaced by hypermutation, wherein said cell has no deleterious mutations in genes encoding paralogues and analogues of the RAD51 protein.
- 5 2. The cell according to claim 1, wherein the cell contains wild-type homologous recombination activity.
3. The cell according to claim 1 or 2, wherein the cell has an unaffected proliferation rate.
4. The cell according to any one of claims 1 to 3, wherein the cell is DNA repair proficient.
5. The cell according to any one of claims 1 to 4, wherein the cell is an immunoglobulin-expressing
10 B cell.
6. The cell according to any one of claims 1 to 5, wherein the cell is derived from chicken, sheep, cow, pig or rabbit.
7. The cell according to any one of claims 1 to 6, wherein the cell is a chicken Bursal lymphoma cell.
- 15 8. The cell according to any one of claims 1 to 7, wherein the cell is a DT40 cell or a derivative thereof.
9. The cell according to any one of claims 1 to 8, wherein the cell expresses activation-induced deaminase (AID).
10. The cell according to any one of claims 1 to 9, wherein the cell is capable of directed and
20 selective genetic diversification of a target nucleic acid by hypermutation or a combination of hypermutation and gene conversion.
11. The cell according to claim 10, wherein the target nucleic acid encodes a protein or exercises a regulatory activity.
12. The cell according to claim 11, wherein the target nucleic acid encodes an immunoglobulin
25 chain, a selection marker, a DNA-binding protein, an enzyme, a receptor protein, or a part thereof.
13. The cell according to claim 12, wherein the target nucleic acid is a human immunoglobulin V-gene or a part thereof.

14. The cell according to claim 11, wherein the target nucleic acid contains a transcription regulatory element or an RNAi sequence.
15. The cell according to any one of claims 10 to 14, wherein the cell further contains at least one sequence capable of serving as a gene conversion donor for the target nucleic acid.
- 5 16. The cell according to any one of claims 10 to 15, wherein the target nucleic acid is integrated into the chromosome at a defined location by targeted integration.
17. The cell according to any one of claims 11 to 16, wherein the target nucleic acid is operably linked to control nucleic acid sequences that direct genetic diversification.
18. The cell according to any one of claims 11 to 17, wherein the cell expresses the target nucleic acid in a manner that facilitates selection of cells comprising mutants of said nucleic acid
10 having a desired activity.
19. The cell according to claim 18, wherein the selection is a direct selection for the activity of the target nucleic acid within the cell, on the cell surface or outside the cell.
20. The cell according to claim 18, wherein the selection is an indirect selection for the activity of a
15 reporter nucleic acid.
21. The cell according to any one of claims 10 to 20, wherein genetic diversification of the target nucleic acid by gene conversion and hypermutation is modulated by genetic manipulation.
22. The cell according to claim 21, wherein the modulation is by cis-acting regulatory sequences.
23. The cell according to claim 20 or 22, wherein the modulation is by varying the number, the
20 orientation, the length or the degree of homology of the gene conversion donors.
24. The cell according to any one of claims 20 to 23, wherein the modulation is by a trans-acting regulatory factor.
25. The cell according to claim 24, wherein the trans-acting regulatory factor is activation-induced deaminase (AID).
- 25 26. The cell according to claim 24, wherein the trans-acting factor is a DNA repair or recombination factor other than a RAD51 paralogue or analogue.
27. A cell line derived from the cell of any one of claims 1 to 26.

28. A non-human transgenic animal containing a lymphoid cell having gene conversion fully or partially replaced by hypermutation, wherein said cell has no deleterious mutations in genes encoding paralogues and analogues of the RAD51 protein, and wherein said cell is capable of directed and selective genetic diversification of a transgenic target nucleic acid by hypermutation or a combination of hypermutation and gene conversion.
29. A method for preparing a cell capable of directed and selective genetic diversification of a target nucleic acid by hypermutation or a combination of hypermutation and gene conversion comprising (a) transfecting a lymphoid cell capable of gene conversion with a genetic construct containing the target nucleic acid, and (b) identifying a cell having the endogenous V-gene or a fragment thereof replaced with the target nucleic acid.
30. The method according to claim 29, wherein the genetic construct containing the target nucleic acid further contains at least one nucleic acid capable of serving as a gene conversion donor for the target nucleic acid.
31. The method according to claim 29 or 30, wherein the locus containing the target nucleic acid is constructed by multiple rounds of transfection.
32. The method according to any one of claims 29 to 31 further comprising (c) transfecting the cell from step (b) with a further genetic construct comprising a reporter gene capable of being influenced by the target nucleic acid.
33. The method according to any one of claims 29 to 32 further comprising (d) conditional expression of a trans-acting regulatory factor.
34. The method of claim 33, wherein the trans-acting regulatory factor is activation-induced deaminase (AID).
35. The method according to any one of claims 29 to 34, wherein the target nucleic acid is inserted into the cell chromosome at a particular location by targeted integration.
36. A method for preparing a gene product having a desired activity, comprising the steps of: (a) culturing cells according to any one of claims 11 to 26 under appropriate conditions to express the target nucleic acid, (b) identifying a cell or cells within the population of cells which expresses a mutated gene product having the desired activity; and (c) establishing one or more clonal populations of cells from the cell or cells identified in step (b), and selecting from said clonal populations a cell or cells which express(es) a gene product having an improved desired activity.

37. The method according to claim 36, wherein steps (b) and (c) are iteratively repeated.
38. The method according to claims 36 or 37 further comprising the step of switching off genetic diversification.
39. The method according to any one of claims 36 to 38, wherein the diversification of the target
5 nucleic acid is further modified by target sequence optimization.
40. The method according to any one of claims 36 to 39, wherein the genetic diversification is switched off by down-regulation of the expression of a trans-acting regulatory factor.
41. The method according to claim 40, wherein the trans-acting regulatory factor is activation-induced deaminase (AID).
- 10 42. Use of the cell according to any one of claims 10 to 26 or a cell line according to claim 27 for the preparation of a gene product having a desired activity.